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### Hymenoptera venom allergy

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# Initiating yellow jacket venom immunotherapy with a 100-microgram dose: a challenge?

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## ABSTRACT

**Background:** Although venom immunotherapy (VIT) with partially purified venom (Pharmalgen®) is highly effective in preventing systemic reactions in yellow jacket (YJ) venom allergic patients, its cost-effectiveness has recently been questioned. Up-dosing is particularly expensive while the reductive effect on adverse reactions to VIT has never been demonstrated.

**Objective:** Initiation of YJ-VIT with 100 µg Pharmalgen® was compared to up-dosing with a modified rush regimen in terms of adverse reactions, clinical efficacy, immunological effects and costs.

**Methods:** In this randomized clinical trial, eighteen patients received YJ-VIT initiation by a single injection of 100 µg Pharmalgen® followed by 3 booster injections every 4 weeks, or, alternatively, 12 updose injections in 7 weeks followed by one booster injection 5 weeks later. Adverse reactions were registered. Clinical efficacy was assessed by a sting challenge and compared to sting challenge outcomes in twenty untreated patients. Allergen-blocking IgG capacity was assessed with the ELIFAB assay. Direct medical costs and indirect costs were estimated as actual costs from a societal perspective.

**Results:** No systemic adverse reactions were observed to either VIT regimen and both regimens offered complete protection against systemic reactions to subsequent sting challenges. In contrast, 20% of untreated patients reacted systemically to a sting challenge. Allergen blocking IgG capacity was increased equally by the two VIT regimens. The 100-µg regimen and modified rush regimen cost \$323.76 and \$856.52, respectively.

**Conclusions and clinical relevance:** This is the first indication that YJ-VIT may be initiated at the maintenance dose in selected patients, thereby avoiding time-consuming regimens and reducing costs. Further studies on the safety of this approach are warranted.

## INTRODUCTION

Allergic reactions to Hymenoptera stings range from late-phase large local reactions limited to the site of the sting to immediate systemic reactions with generalized skin, gastrointestinal, respiratory, or cardiovascular system involvement.<sup>1</sup> The prevalence of systemic reactions related to Hymenoptera venom allergy is estimated at 3%, with yellow jacket (YJ) stings accounting for the majority of anaphylactic reactions in adults.<sup>2,3</sup> Without treatment, 25–60% of patients with a YJ venom allergy develop a systemic reaction to an in-hospital sting challenge.<sup>4,7</sup>

The risk of anaphylaxis can be reduced to the general population hazard of 5% by YJ venom immunotherapy (YJ-VIT).<sup>5</sup> Moreover, sting reactions that occur during YJ-VIT are usually milder than those experienced before treatment, and YJ-VIT is highly effective in improving quality of life.<sup>8</sup> One of the most commonly used YJ-VIT products is Pharmedgen®, a partially purified extract of raw YJ venom. Recently, the cost-effectiveness of Pharmedgen® has been questioned based on a cost estimate of \$27.5 million per quality-adjusted life year.<sup>9</sup> Despite the controversy relating to failure of this study to include specific effects on quality of life,<sup>10</sup> it does suggest that critical appraisal of current treatment regimens and exploration of more cost-effective options are warranted.

In terms of non-medical costs, the up-dosing phase is a relatively expensive part of YJ-VIT as its time-consuming nature causes economic productivity losses. The purpose of up-dosing is to reduce the risk of systemic adverse reactions to VIT, and up-dosing has been common practice since immunotherapy with venom extract was first introduced in 1974.<sup>11</sup> However, the reductive effect on systemic adverse reactions has never been demonstrated, and randomized controlled trials on treatment regimens are scarce. Several studies have suggested that not the dose but the exposure rate plays a pivotal role in the incidence of systemic adverse reactions.<sup>12–14</sup> In these studies, rush regimens result in less systemic adverse reactions than conventional regimens, while greater incremental steps are taken using fewer injections. In another study, Hymenoptera venom allergic patients tolerated 1, 10 and 100 µg venom injections given at 30-minute intervals, while 58% developed a systemic reaction to a subsequent sting challenge.<sup>5</sup> Since patients showed no systemic adverse reaction to 100 µg of YJ-VIT, we hypothesized that this dose might be applied as the starting dose when initiating YJ-VIT in selected patients. Elimination of the up-dosing phase would reduce costs, increase patient convenience, and shorten the time to reach clinical protection.

In this randomized controlled pilot study, up-dosing with 4 monthly 100 µg Pharmedgen® injections was compared with up-dosing according to a modified rush regimen, in terms of elicitation of adverse reactions, clinical efficacy (as ascertained by a sting challenge and compared to a group of YJ venom allergic patients not treated with VIT undergoing a sting challenge), immunological effects (measuring allergen-specific antibody profiles and the allergen-blocking capacity of patient sera by the enzyme-linked immunosorbent facilitated antigen binding (ELIFAB) assay), and treatment costs.

## METHODS

### Subjects

This pilot study was an investigator-initiated, prospective, randomized clinical trial conducted at the University Medical Center Groningen between May 2013 and November 2015. Participants had a history of a systemic reaction grade I-IV, classified according to the system proposed by Mueller, following a YJ field sting,<sup>15</sup> in addition to YJ venom-specific (s)IgE > 0.35 kU<sub>A</sub>/L in serum, measured within two months before inclusion. Patients with double sensitization to YJ venom and bee venom were excluded. For safety reasons, patients were prohibited from participation if they met the relative contraindications for YJ-VIT with Pharmedgen® as specified by the manufacturer (ALK-Abelló, Hørsholm, Denmark): severe cardiopulmonary disease, insufficiently controlled asthma, immune deficiencies, auto-immune diseases, malignancy, severe kidney failure, use of  $\beta$ -blockers or immunosuppressive drugs, or pregnancy.<sup>16</sup> Additionally, patients were not included if they were younger than 18 years, experienced a grade IV sting reaction with loss of consciousness or incontinence, or suffered from mastocytosis. Patients were screened for mastocytosis by measuring baseline serum tryptase (cut-off value 10.0  $\mu$ g/L<sup>17</sup>), urine methylhistamine (MH; reference value 154  $\mu$ mol/mol) and methylimidazole acetic acid (MIMA; reference value 1.9 mmol/mol), and inspecting the skin for clinical lesions compatible with urticaria pigmentosa.

The trial was registered at the European Clinical Trials Database (EudraCT no. 2013-001990-26) and was approved by the National Research Ethics Service and the Institutional Review Board of the University Medical Center Groningen. All participants provided written informed consent.

### Treatment and sting challenge regimens

Participants were computerized stratified randomized to one of the two following treatment regimens of YJ-VIT with Pharmedgen® (ALK-Abelló, Hørsholm, Denmark), balanced for age, gender, and severity of field sting reaction (**Table 1**). Regimen 1 encompassed VIT initiation by a single injection of 100  $\mu$ g YJ venom, followed by 3 monthly 100  $\mu$ g injections, resulting in a cumulative dose of 400  $\mu$ g YJ venom in 12 weeks. Regimen 2 resembled a modified rush (cluster) regimen, with a total of 6 injections every 30 minutes on the first day (starting with 0.0001  $\mu$ g, final amount 10  $\mu$ g, cumulative dose 11.1111  $\mu$ g/d), followed by weekly injections of increasing venom doses up to the final amount of 100  $\mu$ g YJ venom at week 7, and a booster injection of another 100  $\mu$ g YJ venom 5 weeks later (cumulative dose 421  $\mu$ g in 12 weeks). The treatment regimens are outlined in **Table 2**.

**Table 1** | Clinical and laboratory patient characteristics.

	Regimen 1 (n = 10)	Regimen 2 (n = 8)	Comparators (n = 20)
Age (years)	43.1 ± 12.4	52.9 ± 5.5	51.25 ± 12.2
Gender (male, %)	6 (60.0)	6 (75.0)	10 (50.0)
Field sting reaction (grade, %) I	1 (10.0)	1 (12.5)	3 (15.0)
II	2 (20.0)	2 (25.0)	3 (15.0)
III	3 (30.0)	1 (12.5)	7 (35.0)
IV	4 (40.0)	4 (50.0)	7 (35.0)
Interval field sting – start VIT or sting challenge (months)	9.7 (8.4–20.6)	9.6 (7.7–10.4)	11.2 (8.5–25.0)
Yellow jacket venom-sIgE (kU <sub>A</sub> /L)	3.51 (1.47–7.22)	7.96 (3.28–16.62)	5.15 (3.06–8.35)
Tryptase (µg/L)	5.0 (3.9–6.7)	5.5 (3.7–6.0)	4.3 (3.5–5.1)
MH (µmol/mol creatinine)	93 (62–125)	113 (73–114)	83 (60–108)
MIMA (mmol/mol creatinine)	1.1 (0.9–1.4)	1.0 (0.9–1.3)	1.4 (1.2–1.6)*

Mean ± SD or median (interquartile ranges). Group differences were tested using the independent samples *t*-test, Mann-Whitney *U*-test, or Kruskal Wallis test. \**P* < .05 for patients on regimen 2 vs. sting protocol. Regimen 1, 4 monthly 100 µg injections; regimen 2, modified rush regimen; comparators, untreated patients receiving a sting challenge.

MH, methylhistamine; MIMA, methylimidazole acetic acid; VIT, venom immunotherapy.

**Table 2** | Injection scheme regimen 1 and regimen 2.

Injection scheme regimen 1			Injection scheme regimen 2		
Week	Injection	Dose (µg)	Week	Injection	Dose (µg)
1	1	100	1	1	0.0001
4	2	100		2	0.001
8	3	100		3	0.01
12	4	100		4	0.1
Final cumulative dose 400 µg				5	1
				6	10
			2	7	10
			3	8	20
			4	9	40
			5	10	60
			6	11	80
			7	12	100
			12	13	100
			Final cumulative dose 421 µg		

To compare clinical efficacy of the VIT regimens, an in-hospital sting challenge (the gold standard) was performed 4 weeks after the last injection of each regimen. This outcome was compared to the outcome of a sting challenge in patients not treated with VIT (hereafter referred to as the comparator group, patient characteristics **Table 1**). Sting challenges were performed according to European guidelines with *Vespula vulgaris* or *Vespula germanica*, confirmed by entomologic determination, and caught in the wild on the morning of the challenge.<sup>18</sup> Stings were performed on the volar side of the arm and local sting reactions (e.g. erythema and local swelling) were measured and recorded after 15 minutes.

As a safety measure, all sting challenges and injections of regimen 1, as well as the first six injections of regimen 2, were administered in an intensive care setting. Patients were under constant medical supervision with intravenous access and monitoring of blood pressure, respiratory and cardiac function, allowing prompt recognition and treatment of anaphylaxis. Adverse reactions were ascertained and documented according to predefined criteria including condition of the skin, pulse rate, blood pressure, and respiratory rate. To objectify suspected systemic reactions, the course of serum tryptase and the urinary histamine metabolites were measured, comparing baseline values to values one and two hours after the first symptoms. Symptoms of late-phase reactions (e.g. large local reactions defined as local swelling with a diameter of > 10 cm) were systematically evaluated using a questionnaire.

### **Hymenoptera venom-specific serum antibodies and mast cell parameters**

YJ venom-sIgE and -sIgG4 antibodies, Ves v 5, and bee venom-sIgE were measured in patients' serum by the ImmunoCAP system according to the manufacturer's instructions (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden). Serum tryptase levels were determined with the B<sub>12</sub> assay using ImmunoCAP Tryptase reagents and the Phadia 250 analysis device (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden).<sup>19</sup> Levels of MH and MIMA were determined by an isotope-dilution mass fragmentographic method as described previously.<sup>20-22</sup>

### **Enzyme-linked immunosorbent facilitated antigen-binding (ELIFAB) assay**

In order to evaluate immunological changes during the initial phase of VIT, blood was drawn immediately before the first Pharmedin<sup>®</sup> injection and immediately before the sting challenge. The allergen-blocking capacity of patients' sera was assessed with the cell-free ELIFAB assay.<sup>23</sup> The blocking capacity depends on allergen-specific IgG antibodies, as neither serum of non-sensitized healthy controls nor IgG-depleted serum of patients treated by VIT inhibits allergen-IgE complex binding to CD23.<sup>24</sup>

In brief, 20 µL of IgG-depleted<sup>25,26</sup> indicator serum exhibiting high Ves v 5-sIgE levels (> 100 kU<sub>A</sub>/L) was either mixed with 20 µL of patient serum or 20 µL medium in the presence of 5 µL recombinant Ves v 5. As YJ venom extract consists of a complex mixture of different allergens, the major allergen Ves v 5 was used as a surrogate to allow defined assay conditions. The indicator serum was previously shown to exhibit an optimal antibody/allergen ratio



for complex formation at 3 µg/mL Ves v 5.<sup>24</sup> The solution was preincubated at 37°C for one hour in order to allow for formation of allergen-IgE complexes. After preincubation, allergen-IgE complexes were transferred to soluble CD23-coated plates (R&D Systems, Wiesbaden-Nordenstadt, Germany) and incubated for one hour at room temperature. Next, free sIgE was washed away and allergen-IgE complexes bound to immobilized soluble CD23 (Sigma-Aldrich, Schnelldorf, Germany) were detected by adding biotin-conjugated anti-human IgE antibody (BD Biosciences, Heidelberg, Germany), streptavidin-peroxidase, and 3,3',5,5'-tetramethylbenzidine (TMB; both Sigma-Aldrich, Schnelldorf, Germany). All samples were measured in duplicate. For the calculation of antibody ratios, any individual value below the detection limit for Ves v 5-sIgG4 was arbitrarily replaced by 0.01 mg<sub>A</sub>/L in statistical analysis.

### Statistical methods

Statistical analysis was performed with IBM Statistics 22.0 (SPSS, Armonk, NY). Categorical variables were expressed as percentage and metric variables as mean or median and standard deviation or interquartile range, respectively. Group differences were tested using the independent samples *t*-test, Mann-Whitney *U*-test, or Kruskal Wallis test. Percentages were compared using the Chi-Square test. *P* values < .05 were considered to be statistically significant.

### Pharmacoeconomic evaluation

All health care costs and non-health care costs were valued from a societal perspective according to standard Dutch guidelines for economic evaluations (Dutch Health Care Insurance Board),<sup>27,28</sup> meaning that costs were expressed as actual costs instead of tariffs. All prices were converted to the price level of 2015 and converted from euro to US dollars. A detailed overview of the cost components and prices implemented per patient per regimen is shown in **Table 3**. In addition to the study regimens, the costs of the conventional up-dosing regimen are shown.<sup>16</sup>

### Health care costs

The Pharmedgen® purchase price is \$26 per 100 µg.<sup>27</sup> The average costs of an outpatient department (OPD) visit were calculated as follows: for each visit, a nurse (salary cost: \$31 per hour) is present for 15 minutes. In general, reactions occur in 1.9% of the updosing injections<sup>29</sup> for which an allergist (salary cost: \$79 per hour) must be present for half an hour and a nurse for two hours. On average, this comes to \$2 per visit; with an additional 44% overhead for the accommodation, cleaning and equipment. In total, this yields a total average cost of \$14 per visit. In addition to the OPD visit costs, a half-day of hospital care during the first day of updosing of regimen 2 was included, as patients are admitted to hospital (\$309 per day) for three hours.<sup>8</sup>

**Table 3** | Overview of costs per regimen per patient.

	Unit costs (US \$)*	Regimen 1	Regimen 2	Conventional regimen
<b>Health care costs</b>				
Pharmalgen	\$26.29 per 100 µg	\$105.16	\$110.68	\$104.14
OPD visit	\$14.01 per injection	\$56.04	\$182.13	\$168.12
Half day hospital care	\$154.29 per visit	–	\$154.29	–
<i>Subtotal health care costs</i>		\$161.20	\$447.10	\$272.26
<b>Non-health care costs</b>				
Travel costs	\$0.24/km*; \$3.56 parking costs	\$27.68	\$55.36	\$83.04
Productivity loss	Hourly wage men \$37.50 (20.50–45.18) Hourly wage women \$29.94 (19.84–33.08)	\$134.88	\$354.06	\$404.64
<i>Subtotal non-health care costs</i>		\$162.56	\$409.42	\$487.68
<b>Total costs</b>		<b>\$323.76</b>	<b>\$856.52</b>	<b>\$759.94</b>

\*Exchange rate January 2016 (€1 = US \$1.09). All prices are derived from the Dutch Health Care Insurance Board. Regimen 1, 4 monthly 100 µg injections; regimen 2, modified rush regimen.

\*Mean travel distances: 7.0 km to specialist

OPD, Outpatient department

### **Non-health care costs**

On average, patients travel 7 kilometers to reach the OPD and pay \$4 for parking at the hospital.<sup>28</sup> Productivity losses were quantified in terms of lost earnings caused by absenteeism. Time spent per visit was half an hour of treatment time, and half an hour of waiting and traveling to the OPD. An average age of 40 years and an equal distribution of men and women were applied, leading to an hourly wage of \$38 for men and \$30 for women.<sup>28</sup>

## **RESULTS**

Ten patients were randomized to regimen 1 and eight patients to regimen 2. The comparator group receiving only a sting challenge consisted of twenty patients. All participants completed the study regimen. Patient characteristics are outlined in **Table 1**. Subjects did not differ in demographic characteristics, grade of the field sting reaction, interval between the field sting and start of VIT or sting challenge, YJ venom-sIgE or mast cell parameters, except for a slightly higher MIMA level in the comparator group compared to the group of regimen 2 (1.4 vs. 1.0 mmol/mol creatinine;  $P = .027$ ). However, all mast cell parameters were within normal range.

## **Adverse reactions to YJ-VIT**

### ***Immediate systemic reactions***

None of the subjects developed a systemic reaction to VIT. One patient on regimen 1 developed tachycardia without any measurable change in blood pressure within 10 minutes of the first, second and third 100 µg injection with a duration of a few minutes. This patient recognized the feeling of palpitations under other circumstances, usually at home and related to emotional stress. One patient on regimen 2 developed tachycardia after 10 µg of YJ venom, without other symptoms of anaphylaxis. However, all mast cell parameters remained stable in both patients.

### ***Late phase reactions***

Fatigue and flu-like symptoms developed repeatedly in four patients on regimen 1 and in four patients on regimen 2, several hours after the injection. On regimen 1, large local reactions appeared in eight out of ten patients after the first YJ venom injection, in seven after the second, in three after the third, and in one after the fourth injection. During the course of VIT, a reduction in local reaction size on the injections was observed. On regimen 2, six out of eight patients developed a large local reaction on the first day, and one patient after each injection until the fifth week of up-dosing.

## **Adverse reactions to the sting challenge**

None of the subjects treated with YJ-VIT developed a systemic reaction after the sting challenge. Of note, two patients on regimen 1 experienced an additional, accidental, YJ sting in the field between the third and fourth injection, but neither of them developed a systemic reaction.

In the comparator group, four out of twenty patients (20%) developed a systemic reaction to the sting challenge consisting of three grade I and one grade III reactions (**Table 4**). All clinically diagnosed systemic reactions were biochemically supported by an increase and subsequent decrease in mast cell parameters compared to baseline values. Two other subjects showed tachycardia without any measurable change in blood pressure and flushing of the head, neck and chest, but the mast cell parameters remained stable.

Table 4 | Overview of clinical and laboratory characteristics of reactors to sting challenges.

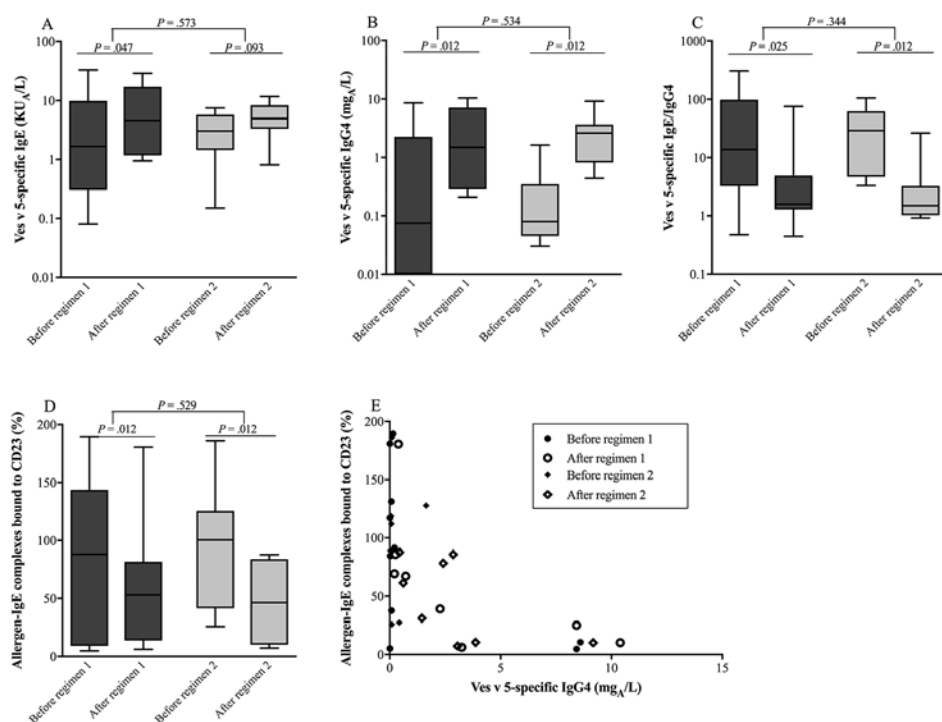
Patient 1		Patient 2		Patient 3		Patient 4	
<b>Symptoms</b>		Within 2 minutes: Feeling of impending doom, anxiety and confusion; metallic taste in mouth; tachycardia; swollen lips and eyes; obstruction nose and red sclera eyes; flushing of head, chest and arms; nausea, abdominal cramping and urge to defecate. Intervention with epinephrine after 3 minutes. Pronounced shaking afterwards.		After 20 minutes: Headache and heavy feeling; flushing of face, neck, chest, and upper part back; urticaria neck, back and abdomen.		After 35 minutes: Increase in blood pressure; tachycardia; headache; swelling of the neck; change in voice; urticaria on neck, chest and back.	
						After 11 minutes: Flushing of face, chest, abdomen and arm; metallic taste in mouth; feeling of deafness; swollen throat with tingling sensation; edema eyes; itchiness all over body.	
<b>Tryptase</b> (µg/L)	Before sting	4.2		4.6		4.5	
	1 hour after sting	18.4		6.2		7.8	
	2 hours after sting	11.2		5.4		4.1	
<b>MH</b> (µmol/mol creatinine)	Before sting	68		96		113	
	1 hour after sting	191		179		681	
<b>MIMA</b> (mmol/mol creatinine)	Before sting	1.0		1.3		1.4	
	1 hour after sting	4.7		1.4		9.9	

MH, methylhistamine; MIMA, methylimidazole acetic acid.

### Hymenoptera venom-specific serum antibodies and ELIFAB

Ves v 5-sIgE antibodies were detectable in all sera and increased equally during regimen 1 and regimen 2 (1.39 and 1.20 kU<sub>A</sub>/L, respectively;  $P = .573$ ; **Figure 1A**). In addition, Ves v 5-sIgG4 antibody concentrations were equally increased (1.23 and 1.87 mgA/L, respectively;  $P = .534$ ; **Figure 1B**). The latter resulted in a comparable decrease of the Ves v 5-sIgE/-sIgG4 ratio, seen in both schedules (12.25 and 15.26, respectively;  $P = .344$ ; **Figure 1C**).

Although addition of some of the patient sera resulted in a greater degree of IgE-allergen interaction than the indicator serum alone, all sera showed an increased ability to inhibit allergen-IgE complex formation after VIT (**Figure 1D**). No difference was found in the decrease of allergen-IgE complex binding to CD23 between the patients during regimen 1 and regimen 2 (47.8% and 37.8%, respectively;  $P = .529$ ). After VIT, an inverse correlation was found between the Ves v 5-sIgG4 antibody concentrations and the percentage of allergen-IgE complexes bound to CD23 (two-tailed Spearman's rank correlation coefficient:  $r_s = -0.833$  vs.  $-0.714$ ;  $P = .01$  and  $.047$ , respectively; **Figure 1E**).



**Figure 1** A and B | Ves v 5-specific (s)IgE and -sIgG4 antibody concentrations; C) Ves v 5-sIgE/IgG4 ratio; D) ELIFAB. Data are related to binding with indicator serum alone (100%); E) Correlation between Ves v 5-sIgG4 antibody concentrations and percentage of allergen-IgE complexes bound to sCD23. Comparisons of paired samples vs. unpaired samples were made using Wilcoxon Signed-Rank test and Mann-Whitney  $U$ -test.

### Pharmacoeconomic evaluation

On regimen 1, the cost per patient was \$324 compared with \$857 on regimen 2, which represents a 2.6 fold reduction (**Table 3**). The main causes for the cost difference were productivity loss (\$219), hospital care costs for the first day of up-dosing (\$154) and OPD visit costs (\$126). Conventional up-dosing costs \$760 per patient, which is 2.3 times more than calculated for the treatment by regimen 1.<sup>16</sup>

## DISCUSSION

Since immunotherapy with Hymenoptera venom extract was first carried out in 1974, up-dosing of venom at the start of VIT has been a standard procedure. It is a widely held view that up-dosing can reduce the occurrence of systemic adverse reactions to VIT. This is the first randomized controlled study exploring the option of VIT initiation without an up-dosing phase but rather by starting with a single dose of 100 µg YJ venom (Pharmalgen®), corresponding to the usual maintenance dose. We demonstrate that a regimen starting with a single dose as high as the maintenance dose of 100 µg/injection is tolerated as well as a regimen with incremental venom doses. Although this study is limited by a small sample size and additional research in a larger number of patients is needed to ensure therapy safety, these results give a first indication that it may be safe to start VIT directly with the maintenance dose of 100 µg/injection in selected patients.

No systemic reactions were observed during the 100 µg YJ venom regimen, or the modified rush regimen. Remarkably, 20% of the patients not treated by VIT reacted systemically to a sting challenge. Our results are in agreement with the study by Hunt et al., in which patients tolerated 1, 10 and 100 µg of purified venom given at 30-minute intervals, but in which 58% reacted systemically to a subsequent sting challenge.<sup>5</sup> These combined data suggest that the composition of Hymenoptera venom extracted for purposes of immunotherapy could be somewhat different from the venom injected by a living insect. Pharmalgen® is obtained from the venom sacs of a mixture of *Vespula* species. After the sac is pulled out of the insect, it is crushed in a β-alanine acetic acid buffer to release the venom.<sup>16</sup> Supposedly, the allergens characterized to date (Ves v 1, 2, 3, 5, and 6)<sup>30</sup> are present in venom after extraction but hitherto unknown allergens may be left behind in the venom sac or lost upon exposure to a non-natural environment. Moreover, little is known about the protein recovery and stability resulting from the extraction process, both of which may alter the final composition of the extract utilized for VIT in comparison to venom found in its natural molecular environment in the venom sac.

All treated patients were clinically protected from systemic reactions to the sting challenge, regardless of the utilized VIT regimen, while 20% of the untreated patients in the comparator group reacted systemically to the sting challenge. Early immunological changes could be demonstrated in both treatment arms as a similar boost in Ves v 5-sIgG4 antibody

concentrations was observed. Additionally, an equally increased ability of patients' serum to inhibit allergen-IgE complex formation after three months of VIT was demonstrated by the ELIFAB assay. This assay was performed in addition to IgG4 measurement, as successful VIT is associated with an increase in IgG4 blocking activity that is more dependent on the affinity than on the quantity of the antibodies.<sup>31</sup> Thus, the non-responsiveness to the sting challenge and development of YJ venom inhibitory IgG antibodies suggest a comparable efficacy of both VIT regimens.

For patient enrolment, strict selection criteria were applied with emphasis on exclusion of patients affected by mastocytosis or suffering from pulmonary or cardiovascular diseases, which result in an increased risk of systemic reactions to VIT.<sup>32,33</sup> Except for patients with mastocytosis, because they have a high risk for severe reactions, future studies should evaluate the safety of directly starting with the maintenance dose of 100 µg YJ venom in these groups of patients. Furthermore, the suitability of this VIT regimen for the treatment of bee venom allergy should be assessed, as bee venom VIT poses a higher risk for systemic adverse effects.<sup>34</sup>

Given the current focus on cost and convenience in health care worldwide, this report may contribute to greater use and acceptance of this important and effective treatment. Starting directly with 100 µg YJ venom brings a 2.6 fold reduction compared to modified up-dosing, or a 2.3 fold reduction compared to conventional up-dosing. Given a prevalence of 3% of systemic reactions to YJ stings,<sup>3</sup> a life expectancy of 79 years,<sup>35</sup> and a mean age of occurrence of 40 years, the calculated incidence of systemic reactions is 0.077%. In a population comparable to that of the United States (323 million people), implementation of regimen 1 in, for example, 50% of the YJ venom allergic patients would save \$54 million compared to conventional up-dosing each year. Costs were analyzed according to standard Dutch guidelines for economic evaluations. In the US setting, health expenditures are higher than in the Netherlands. The total health expenditure per capita is \$8.713 in the US compared to \$5.217 in the Netherlands,<sup>36</sup> implying that the cost difference between the regimens would be even greater in the US.

In conclusion, the results of this study give a first indication that VIT initiation at the maintenance dose may be safe, clinically effective, induces YJ venom-blocking IgG antibodies, and is pharmaco-economically advantageous compared to an up-dosing treatment schedule. However, caution is warranted in the selection of eligible patients. As we applied very strict exclusion criteria, with an emphasis on mastocytosis, our findings cannot be extrapolated to all YJ venom allergic patients. Moreover, additional research including a larger number of patients is needed to ensure safety of this VIT regimen. Our results confirm that sting challenges with living insects remain the only test for evaluating clinical allergic reactivity to stinging insects.

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